University *of Ljubljana*
Faculty *of Mathematics and Physics*

Seminar – 4. letnik

NANOMEDICINE: A WAY OF TARGETING AND DETECTION OF CANCER CELLS

Author: Maja Požar

Mentor: prof. dr. Rudolf Podgornik

Ljubljana, November 2010

ABSTRACT

Nanotechnology promises changes in many significant areas of medicine, material science, construction, etc. In medicine advanced nanostructures, such as functional nanoparticles, dendrimers, fullerenes, carbon nanotubes and semiconductor nanocrystals have been exploited for drug delivery, diagnostics and treatment of diseases at the molecular level. Nanoparticles have been developed to improve contrast and allow imaging of target tissues using conventional diagnostic equipment. In this paper we focused mostly on how nanotechnology can change the aspect of targeting and detection of cancer cells and consequently its treatment. Rapid advances in the study of the interface of nanomaterials with biomolecules have led to the programmed self-assembly of nanoparticles through the use of biomolecules. This will result in preserving and improving human health, using molecular knowledge of the human body on nanoscale.

INDEX

INTRODUCTION

Nanotechnology has gained ground in the twenty-first century and is rapidly growing due to the ability to manipulate and harness properties of assemblies that are at the nanosize scale of biomolecules. Bionanotechnology is defined by science's growing ability to work at the molecular level, atom by atom, combining biological materials and the rules of physics, chemistry and genetics to create tiny synthetic structures [1]. The end result of nanotechnology is to create a highly functional system of biosensors, electric circuits, nanosized microchips, molecular "switches" and even tissue analogs for growing skin, bones, muscles, and other organs of the body. All accomplished in ways that allow these structures to assemble themselves, molecule by molecule. Nanostructures, such as functional nanoparticles, dendrimers, fullerenes, carbon nanotubes and semiconductor nanocrystals including quantum dots have been exploited for drug delivery, diagnostics and treatment of diseases at the molecular level. Diseases can be identified based on anomalies at the molecular level, and treatments are designed based on activities in such low dimensions. Although a multitude of methods for disease identification as well as treatment already exists, it would be ideal to use research tools with dimensions close to the molecular level to better understand the mechanisms involved in the processes. These tools can be nanoparticles, designed to examine a biochemical process of interest. Typical dimensions of biomolecular components are in the range of 5 to 200 nm, which is comparable with the dimensions of man-made nanoparticles [1]. By developing nanosized bioparticles that has no or very little effect on human health, diagnostic imaging could also be improved. The possibility of cell-targeted treatment and cancer therapy is expected to emerge from many research groups.

WHAT IS NANOMEDICINE?

Specific application of nanobiotechnology to nano and molecular scale design of devices for the prevention, treatment, and cure of illness and disease is called nanomedicine. Most broadly, nanomedicine is the application of nanotechnology to medicine [2]. It covers process of diagnosing, treating and preventing disease and traumatic injury, relieving pain, preserving and improving human health, using molecular knowledge of the human body on nanoscale.

The early concept of nanomedicine sprang from the visionary idea that tiny nanorobots could be designed and introduced into the human body to perform repairs at the molecular level. This early visionar was Richard P. Feynman, a Nobel physicist, who worked on the Manhattan Project at Los Alamos during World War II. He said: “There’s plenty of room at the bottom.”, suggesting that there is so much potential for exploring physics on the atomic level. In fact in 1959 he suggested a possibility for relatively small machines: “Although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and looks around. It finds out which valve is the faulty one and takes a little knife and slices it out.” [2].

The ability to structure materials and devices at the molecular scale has the potential to revolutionize the future practice of medicine. The majority of work in the area can be classified in one of the following areas:

- therapeutic delivery systems with the potential to deliver genes and pharmaceuticals through specific pathways,
- new biomaterials and tissue engineering for active tissue regeneration,
- biosensors, biochips and new imaging techniques for the purposes of diagnostic monitoring and imaging.

When taking the view of the benefits of nanotechnology in a certain field, we always look at the product performance. In nanoworld products usually have unique properties (biocompatibility, electronic, magnetic, optical, mechanical, thermal, etc.) that are often regarded as contradictory in the “non-nano” world. An example of such a desirable nanomaterial might be a polymer nanocomposite that is mechanically strong and tough (will not bend or break), hard (resists scratching), electrically conductive, optically transparent, and biocompatible. Nanotechnology can result in properties that would be difficult to obtain in the “normal” world. The time to commercialize nanotechnology in certain medical field can be long, considering the development and testing. But the impact that nanotechnology can make in medical devices is unimaginable:

Improved catheters, implants: polymer nanocomposites to improve strength, stiffness and toughness

Joint prostheses: metallic alloys – nanograined, composites and coatings for strength, toughness and wear resistance

Biocompatible surfaces and drug delivery coatings: nanostructured surfaces

Diagnostic and imaging: nanoparticles and carbon nanotubes, implantable biosensors and active muscle, nerve, tissue interfacting electrodes, small, low-power processors with wireless communications

Targeted drug delivery and cancer therapy: nanoparticles

NATURE OF THE NANOWORLD

Nanomaterials can be simply defined to have three features: 1 to 100 nm in one dimension, functional in applications, and easy to manufacture. They also have to be biologically engineered for medical applications. From a biological point of view, the characteristic dimensions of proteins, DNA, polysaccharides, lipids, and viruses fall into this length scale (Figure 1).

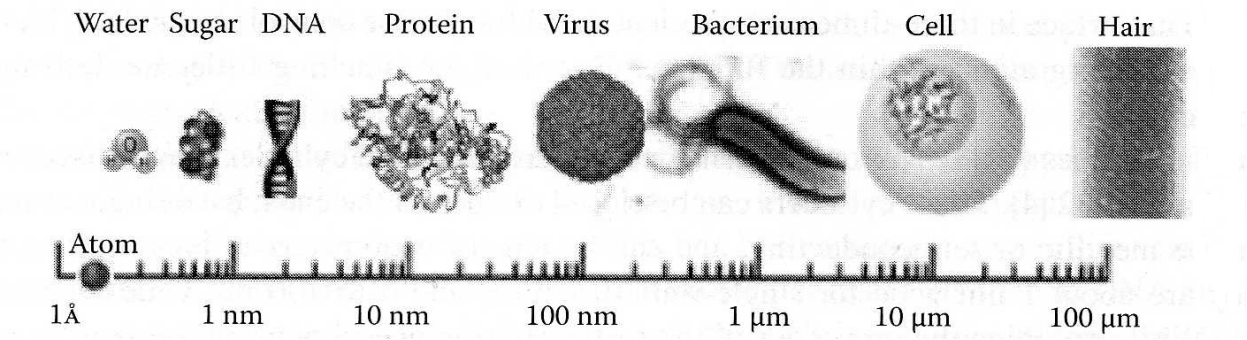
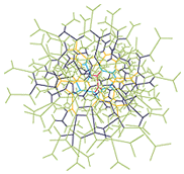
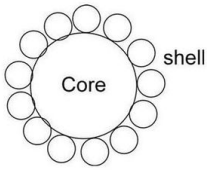
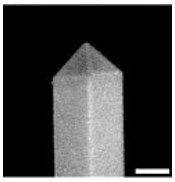


Figure 1: Nanoscale and microscale materials and systems [1].

Living systems are build upon from molecular materials or nanostructures such as nucleid acids (DNA and RNA) and protein. They are about 5 to 50 nm wide and can be produced from the self-assembly or self-organisation processes in the living system or by chemical synthesis. A white blood cell is about 10 μm big, whereas all materials internalized by cells are smaller than 100 nm. The size domain of nanomaterials is similar to that of the biological structures.

These nanostructures have significantly different properties from bulk or microstructures and they are especially suitable for biomedical applications. Here we present a brief introduction to some biologically functional nanomaterials and their biomedical applications:

	*Fullerenes*, C_{60}, 0.7 nm, functional drug carrier with linked antibodies and other targeting agents on the surface carbon atoms and implanted medical devices.
	*Dendrimers*, 5 – 50 nm, branched structure allows to link labels and drugs individually for drug carrier, implanted sensors and medical devices.
	*Nanoparticles*, less than 100 nm, inorganic or organic, for implanted materials, nanoshells and for drug delivery; quantum dots (< 10 nm) and magnetic nanoparticles for labeling and diagnostics and implanted sensors and medical devices.
	*Carbon nanotubes*, 10 – 100 nm, implanted sensors and medical devices
	*Single crystal nanowires*, 5 – 100 nm, one-dimensional nanoparticles, capable of doing what nanoparticles and carbon nanotubes can do.

NANOPARTICLES IN CANCER DIAGNOSIS AND TREATMENT

In cells, proteins are nanomachines that act as transporters, actuators, and motors. They are responsible for specific monitoring and repair processes. In this role nanoparticle is usually a nano-sized polymeric colloidal particle with the drug either encapsulated within the polymer or conjugated onto its surface.

Nanoparticle-based therapeutics – nanopharmaceuticals – are colloidal particles of 10 to 1000 nm (1 micron) that are diverse in size, shape and composition. Therapeutic delivery systems are designed to deliver a range of therapeutic agents, including drugs, proteins, vaccine and plasmid DNA for gene therapy, by exposing target cells to their payload. This requires the

carrier to enter cells where, once internalized, the therapeutic agent is released through vehicle degradation and diffusion mechanisms. The ideal delivery system would be targeted and precisely controlled. Current nanoscale delivery systems are divisible into two major categories: surface modification systems designed to prevent immune response or promote cell growth, and particle-based systems designed to deliver therapeutics to cell and tissues. Particle-based systems include viral carriers, organic and inorganic nanoparticles and peptides. The major advantage of using extra small nanocarriers for delivery is that they can easily penetrate and flow in all size blood vessels and be taken into cells. However, ultrasmall size causes low drug loading capacity and complicated preparation procedures.

There are a few key biological requirements for nanopharmaceuticals to fulfill [3]:

- they must exhibit “stealth” qualities to evade the immune response
- be nontoxic and traceable
- be biodegradable
- they must be selective to be effective in targeting specific tissue.

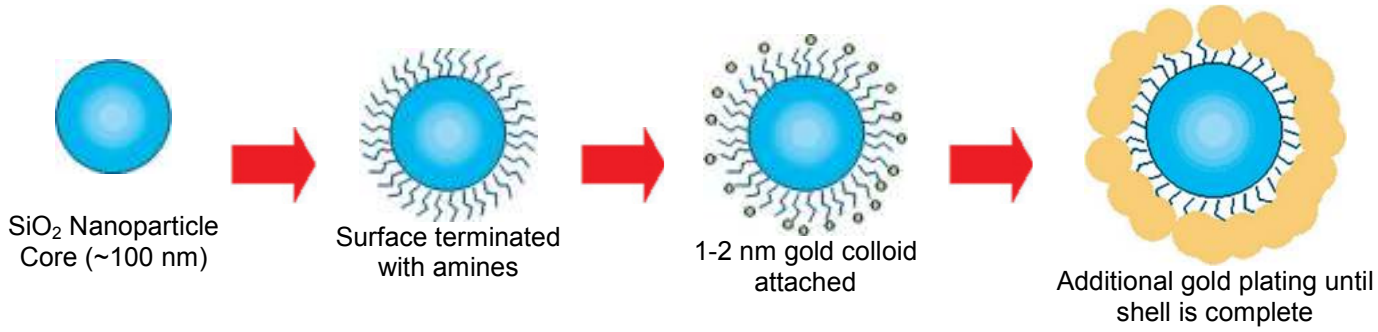
The challenges for site-specific delivery are the cellular barriers, which prevent the drug from reaching the desired target. That is why the science has a need for presenting a carrier system that will overcome this effect. Polymers, used to make nanoparticles, can be divided in two classes: natural and synthetic [1]. Natural polymers, such as proteins and polysaccharides are nontoxic and can easily be biodegraded. However, they are not widely used to make nanoparticles because they vary in purity and often require major optimization before successful encapsulation of drugs can be achieved. Currently, synthetics polymers are frequently used, because they are easy to produce and it is possible to generate degradable nanoparticles with polymers that have accessible surface functional for targeting. A common concern with synthetic polymers is the inability of cells to adequately metabolize the polymer vehicles and ingredients that may be used in their fabrications.

The major advantage of nanoparticulate drug delivery systems is that the release of the active agent can be controlled by the degradation of the polymer nanoparticle shell. It also localizes the target, the delivery is intracellular, it can extend delivery periods, decrease drug dosages and side effects, plus the patient compliance and comfort is improved. Although the application of nanoparticles for targeted drug delivery is well received, much work still needs to be done in research. Understanding the biological processes expressed on infected cells, will improve the development of targeted nanoparticles for drug delivery. These polymeric nanosystems will enable entry and retention of the medicaments in the cells. In addition to these advantages, the carrier systems will reduce unwanted systemic side effects associated with conventional treatment.

One of new findings for nanoscale drug delivery in diagnosing and treating cancer are nanoshells – gold-coated silica [2]. These nanoshells, set in a drug-containing tumor-targeted hydrogel polymer, injected into the body, accumulate near tumor cells. When heated with an infrared light, the nanoshells selectively absorb a specific infrared frequency, melting the

polymer and releasing the drug payload at a specific site. They are designed for specific targeting micrometastases, tiny aggregates of cancer cells too small to remove with a scalpel.

A nanoshell is made up of a few million atoms forming silica particles, essentially tiny glass particles. A chemical is introduced to the nanoshell that enables researchers to attach gold metallic deposits that ultimately form a gold shell around the glass core (Figure 2). In many articles from the last few years, we have noticed several layer-by-layer assembly strategies as engineering approach toward nanoparticles with multilayer shells.



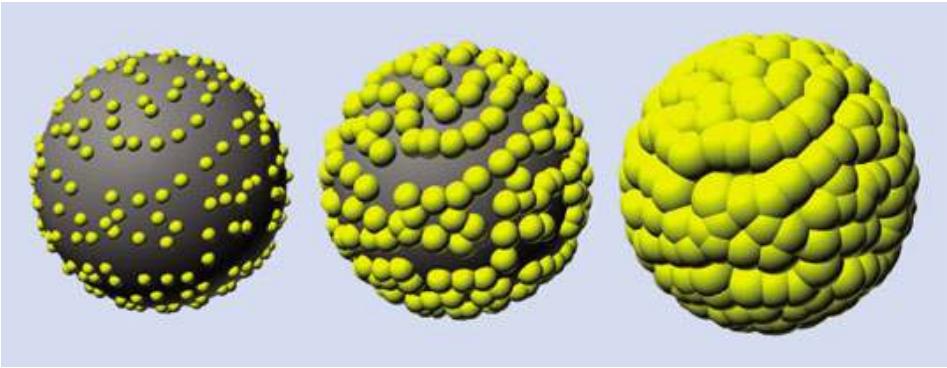


Figure 2: Gold nanoshell synthesis [4].

The key innovation with nanoshells is the ability to tune their color from the visible using the dimensions of the core and the shell to different regions of the spectrum. Therefore, it can tune away from the visible region and into the near-infrared region of the spectrum. Doctors are then able to heat up very precise targeted areas of interest such as tumors. So by using an infrared light from the surface of the skin it can penetrate up to 10 cm into the body. It heats up the tumor eradicating it and passing through the body as waste (Figure 3).

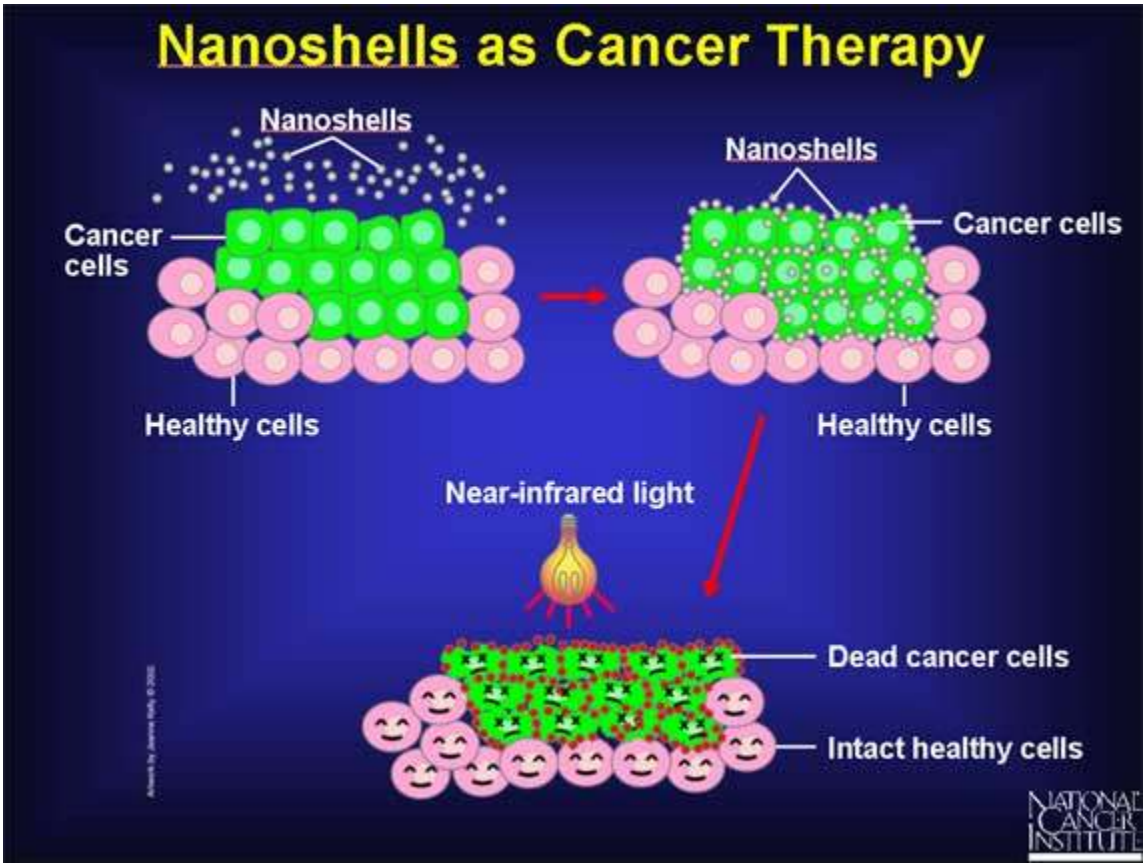


Figure 3: Nanoshells as cancer therapy [5].

In figure 4 we can see the effects of using nanoshells [6]. After laser exposure of 35 W/cm^2 for 7 minutes, all cells within the laser spot underwent photothermal destruction. On the left picture we can see carcinoma cells after exposure to laser, in the middle we can observe carcinoma cells incubated with nanoshells but not exposed to laser light. On the right picture we can see cells incubated with nanoshells after laser exposure. The dark circle seen in the image corresponds to the region of cell death caused by exposure to laser light after incubation with nanoshells [6].

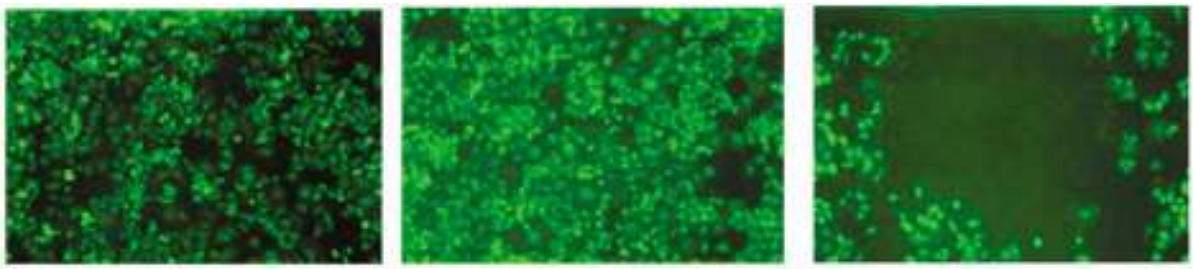


Figure 4: Visible effects of nanoshells interacting with carcinoma cells [6].

Another nanoscale drug delivery system can be created with the use of dendrimers. Dendrimer is precisely constructed molecule built on the nanoscale in a multistep process through up to ten generations and from 5 to 50 nm in scale (Figure 5). A dendrimer is typically symmetric around the core, and often adopts a spherical three-dimensional morphology. Drugs and recognition molecules can be attached to their ends or placed inside cavities within them.

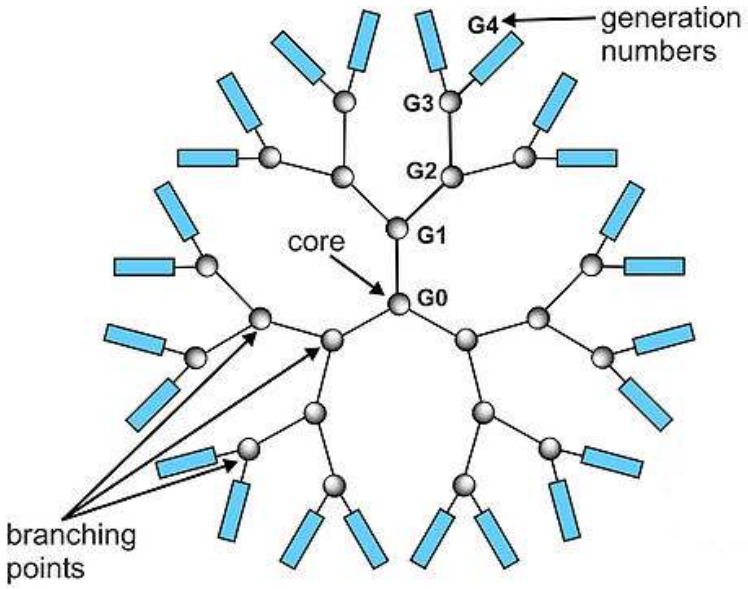


Figure 5: The “growth” of a dendrimer [7].

Dendrimers are versatile, with discrete numbers and high local densities of surface functionalities in one molecule, which is very suitable for cancer therapy, where the disease has multiple stages. The dendritic multifunctional platform is ideal to combine various functions like imaging, targeting, and drug transfer into the cell. The physical characteristics of dendrimers, water solubility, encapsulation ability, and large number of functional peripheral groups, make these macromolecules appropriate candidates for drug delivery vehicles. There are three methods for using dendrimers in drug delivery: first, the drug is covalently attached to the periphery of the dendrimer to form dendrimer prodrugs, second the drug is coordinated to the outer functional groups via ionic interactions, or third the dendrimer acts as a unimolecular micelle by encapsulating a pharmaceutical through the formation of a dendrimer-drug assembly. The dendrimer enhances both the uptake (diffusion or osmosis) and retention of compounds within cancer cells. The encapsulation increases with dendrimer generation and this method may be useful to entrap drugs with a relatively high therapeutic dose.

Dendrimers could also be used to deliver pieces of DNA to the required parts of a cell. Current research is being performed to find ways to use dendrimers to traffic genes into cells without damaging or deactivating the DNA [8].

Quantum dots can technically also be included with nanoparticles, though their properties place them in their own category. Quantum dots are semiconductor nanocrystals (diameter 2 to 10

nm) that exhibit broad excitation spectra and narrow emission spectra in the visible range. Originally designed for information technology purposes, their application for medical imaging with biomolecules was quickly realized [1]. They are capable of confining a single electron in which the electrons occupy discrete energy states just as they would in an atom. These particles show optical gain and stimulated emission at room temperature. They are suitable for biological markers, drug delivery and implanted sensing and heating devices through external lighting. In addition, quantum dots do not fade when exposed to ultraviolet light, and stability of their fluorescence allows longer periods of observation. They demonstrate significant advantages over classic fluorescent dyes, including size-tunable emission wavelength, increased stability, reduced toxicity and persistent residency in cells. Quantum dots do not interfere with cell viability, growth or differentiation over extended periods of time. Their persistence in cells makes them compatible for following extended tissue development, including embryo development. Quantum dots are used for live imaging of tumors, capillaries, skin and adipose tissue. The interest in research of quantum dots increases, so it is possible to develop some kind of identification of “disease fingerprints” [9].

A combinatorially large number of smart therapeutic nanodevices can easily be synthesized from a library of dendrimeric components performing the following tasks:

1. disease cell recognition
2. diagnosis of disease state
3. drug delivery
4. location reporting
5. reporting outcome of therapy.

This framework structure can be produced to fight a particular cancer simply by creating a nanodevice customized to destroy a specific cancer type and no other, while also sparing the healthy normal cells. But first it is necessary to identify proteins unique to each kind of cancer, so that targeting dendrimers could use to identify the cell as cancerous.

METHODS WITH POTENTIAL FOR CANCER THERAPY

To address different diagnostic and treatment requirements in drug delivery system we must use nanoparticle with multilayer shells that combine all of the following properties [10]:

- a small sized nanoparticle carrier
- a high stability in physiological media
- attachment of a drug
- low toxicity of a carrier system
- the triggered release and activation of the drug only
- “stealthiness” and capability to avoid immune response

Several nanoparticles, including gold nanoparticles, quantum dots, and magnetic nanoparticles have been considered for therapeutic or diagnostic purposes, despite the fact that they are still

limited by several factors such as potential aggregation of the nanoparticles in physiological media, their short circulation time or significant uptake by the liver before reaching any target. That is why most cancer treatments are under development, only a few of the methods actually reaches the pre-clinical or clinical trial stage.

One treatment involves targeted chemotherapy that delivers a tumor-killing agent called tumor necrosis factor alpha (TNF) to cancer tumors [11]. TNF is attached to a gold nanoparticle along with Thiol-derivatized polyethylene glycol (PEG-THIOL) known as a chemotherapy drug, which hides the TNF bearing nanoparticle from the immune system. This allows the nanoparticle to flow through the blood stream without being attacked.

An intriguing targeted chemotherapy method uses one nanoparticle to deliver the chemotherapy drug and a separate nanoparticle to guide the drug carrier to the tumor. First gold nanoparticles circulating through the bloodstream exit where the blood vessels are leaking at the site of cancer tumors. Once accumulated at the tumor they are used to concentrate the heat from infrared light; heating up the tumor. This heat increases the level of a stress related protein on the surface of the tumor. The drug carrying nanoparticle (a liposome) is attached to amino acids that bind to this protein, so the increased level of protein at the tumor speeds up the accumulation of the chemotherapy drug carrying liposome at the tumor. Targeted heat therapy is being developed to destroy breast cancer tumors.

X-ray therapy may be able to destroy cancer tumors using a nanoparticle called nbtxr3 [11], which is patented nanoparticle, manufactured by Nanobiotix company, Paris, France. These nanoparticles, when activated by x-rays, generate electrons that cause the destruction of cancer tumors to which they have attached themselves. This is intended to be used in place of radiation therapy with much less damage to healthy tissue.

Delivery of short interfering RNA is another promising way of treating cancer. It is interesting because RNA simply stops the cancer tumor from growing and there is the potential to tailor synthetic (siRNA) to the version of cancer in an individual patient [11]. But still a lot of work needs to be done in research.

THE NANOPARTICLE – HUMAN BODY INTERACTION

Air pollution is already known to cause lung diseases (granulomatosis, silicosis, asbestosis) and potential for cancer. It is a well-known fact that the air polluted by particulate matter size of 10 μm or less is hazardous to our health. Those foreign bodies can enter our system mainly through ingestion or respiration. The particles in size less than 2.5 μm are even more dangerous, as they can cross alveolus barrier and enter the blood stream. In cancerogenic tissue the particles are mainly metals, with the size of debris ranged from tens of nanometers to

10 μm. The chemical compositions of the particles detected in cancer are mostly in unusual combinations and cannot be generalized (Figure 10).

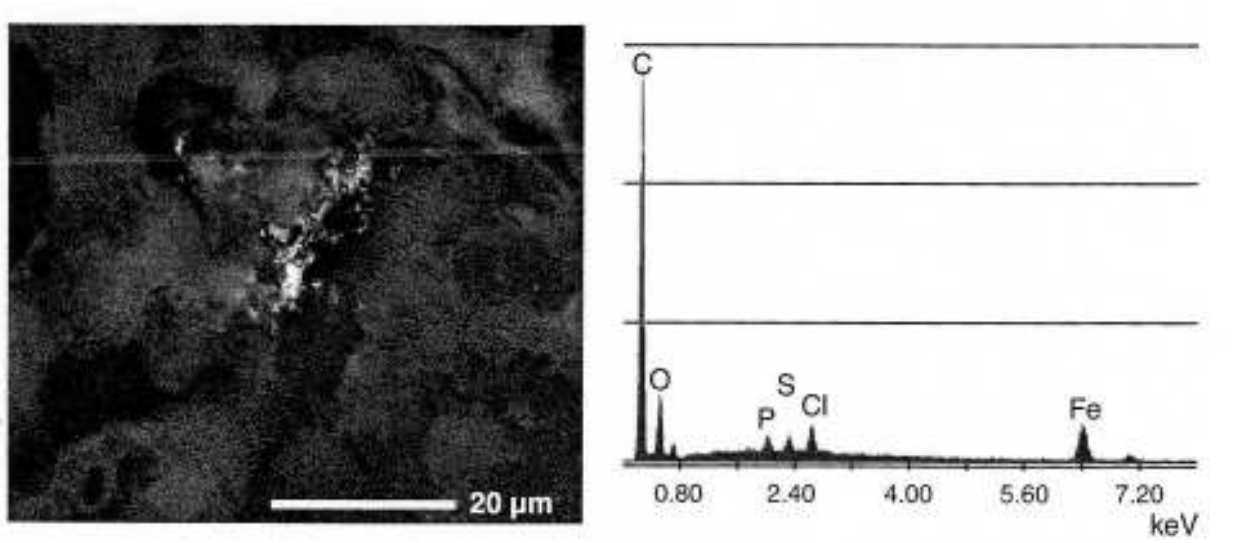


Figure 10: Liver tissue affected by cancer. Clusters of nanoparticles of iron are visible [12].

So what about the nanoparticles that we use for treating cancer?
Medical devices employ biocompatible materials, mainly materials that must be accepted by the biological environment (human body) and induce a favorable biological interaction. There are many cellular processes which are triggered by the type of protein adsorbed. If the presence of a certain protein is requested to guarantee a proper interaction of an implant with the biological surrounding, it may be possible to manipulate the implant surface – examples of that are the molecular self-assembly. Polymers do not activate the defense mediators. They are seen as foreign bodies by cells, but no surface sensors are activated. It was also discovered that metals are more dangerous for the survival activity of the cells [12]. If those bodies are not biodegradable, they induce a reaction through which the organism defends itself against that form of invasion. They can be chemically toxic. The acute toxicity of gold nanoparticles toward human cells can be considered negligible, although toxicity can be very individual. It depends on many parameters, like particle size, concentration, etc. which can be different for each individual.

Other side-effects in treating cancer may occur because of chemotherapy. Some anticancer drugs may affect cells of vital organs, such as the heart, kidney, bladder, lungs, and nervous system. One of the first chemotherapy drugs given to patients diagnosed with cancer (especially lung, ovarian or breast cancer) is cisplatin, a platinum-containing compound that gums up tumor cells DNA. Cisplatin does a good job of killing the tumor cells, but it can also seriously damage the kidneys, which receive high doses of cisplatin because they filter the blood [13].

CONCLUSION

The benefits of nanotechnology are not limited to any particular field of nanomedicine. Areas that will be revolutionized include detection and analysis, drug delivery and biocompatible materials in prosthetic and reconstructive surgery. On the other front, the rapid advances in the study of the interface of nanomaterials with biomolecules have led to the programmed self-assembly of nanoparticles through the use of biomolecules such as DNA, proteins and living cells.

While medical science continues to make significant progress in the treatment of cancer and most major diseases, early diagnosis remains the single most important contributor to therapies. The ability to detect cancerous growths in the body before the cells multiply and spread is critical to effective intervention and prevention.

The incidence rates of cancer of the brain and bladder and melanoma of the skin in women, and testicular cancer in men, are rising (reports from National Cancer Institute 2010, www.ca-medicalnews.com). The potential benefits of nanoparticle cancer treatment are highly acknowledged. Preclinical studies have demonstrated that it is effective and causes no detectable systemic toxicity. Additionally, this therapeutic "device" could increase the effectiveness of standard chemotherapy and radiation. Nanotechnology promises to make significant contributions to the animal and human health sector in both diagnosis and therapy.

REFERENCES

[1] David E. Reisner, *Bionanotechnology: global prospects*, CRC Press, Taylor & Francis Group, Boca Raton, 2009

[2] Robert A. Freitas Jr, *What is Nanomedicine*, Nanomedicine: Nanotechnology, Biology, and Medicine 1, Elsevier Inc. (2005), 2-9

[3] Raj Bawa, *NanoBiotech 2008: Exploring global advances in nanomedicine*, Nanomedicine: Nanotechnology, Biology, and Medicine 5, Elsevier Inc. (2009), 5-7

[4] http://mrsec.wisc.edu (September 2010)

[5] http://jessdea.wordress.com/2010/04 (September 2010)

[6] Christopher Loo, Alex Lin, Leon Hirsch, Min-Ho Lee, Jennifer Barton, Naomi Halas, JenniferWest, Rebekah Drezek, *Nanoshell-Enabled Photonics-Based Imaging and Therapy of Cancer,* Technology in Cancer Research & Treatment, Volume 3, Number 1, Adenine Press, 2004

[7] http://en.wikipedia.org (September 2010)

[8] James C. Mitchell, J. Robin Harris, Jonathan Malo, Jonathan Bath, Andrew J. Turberfield, *Self-Assembly of Chiral DNA Nanotubes*, American Chemical Society (2004), 16342-16343

[9] Edward L. Wolf, *Nanophysics and Nanotechnology: an introduction to modern concepts in nanoscience,* 2nd edition, Wiley – VCH Verlag GmbH & Co. KGaA, Weinheim, 2006

[10] Gregory F. Schneider, Vladimir Subr, Karel Ulbrich, Gero Decher, *Multifunctional Cytotoxic Stealth Nanoparticles. A Model Approach with Potential for Cancer Therapy*, Nano Letters, Vol. 9, No. 2, American Chemical Society (2009), 636-642

[11] http://www.understandingnano.com (September 2010)

[12] Antonietta M. Gatti, Stefano Montanari, *Risk Assessment of Microparticles and Nanoparticles and Human Health*, Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology, Edited by H. S. Nalwa, Volume 2, American Scientific Publishers (2005), 347-368

[13] http://www.nanotechnology-now.com (September 2010)